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DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

147-191P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/485187

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PRIORITY DATE CLAIMED

PCT/EP98/04878

August 5, 1998

August 7, 1997

TITLE OF INVENTION

PROCESSES FOR INCREASING THE YIELD IN PLANTS

APPLICANT(S) FOR DO/EO/US

KWART, Marion; RIESMEIER, Jorg; WILLMEIER, Alois

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(c)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(c) and PCT Articles 22 and 39 (1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
- a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☒ has been transmitted by the International Bureau.
- c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(3)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
- a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☐ have been transmitted by the International Bureau.
- c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
- d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98/-1449 and International Search Report (PCT/ISA/210)
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
- 1.) International Preliminary Examination Report (PCT/IPEA/409) With 3 Amended Sheets
 - 2.) PCT Request Form (PCT/RO/101)
 - 3.) Seventeen (17) sheets of Formal Drawings

INTERNATIONAL APPLICATION NO.

ATTORNEY'S DOCKET NUMBER

147-191P

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):

Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO..... **\$970.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	\$840.00
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International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.	\$690.00
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International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	\$670.00
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International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....	\$96.00
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ENTER APPROPRIATE BASIC FEE AMOUNT =

Surcharge of **\$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

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420 Rec'd PCT/PTO 07 FEB 2000

PATENT
147-191P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: KWART, Marion et al
Int'l. Appl. No.: PCT/EP98/04878
Appl. No.: New Group:
Filed: February 7, 2000 Examiner:
For: PROCESSES FOR INCREASING THE YIELD
IN PLANTS

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION

Assistant Commissioner for Patents
Washington, DC 20231

February 7, 2000

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/EP98/04878 which has an International filing date of August 5, 1998, which designated the United States of America.--

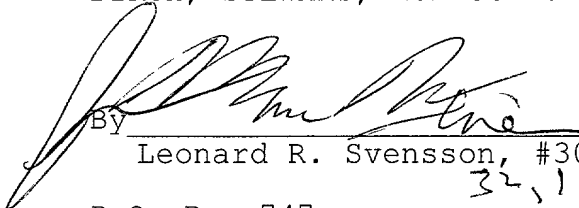
REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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(Rev. 01/08/2000)

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Processes for increasing the yield in plants

Description

The present invention relates to processes for increasing the yield in plants, recombinant nucleic acid molecules used for these processes, their uses as well as to plants with an increased yield.

In the field of agriculture and forestry constantly efforts are being made to produce plants with an increased yield, in particular in order to guarantee the supply of the constantly increasing world population with food and to guarantee the supply of reproducible raw materials. Conventionally, it is tried to obtain plants with an increased yield by breeding, which is, however time-consuming and labor-intensive. Furthermore, appropriate breeding programs have to be performed for each relevant plant species.

Progress has partly been made by the genetic manipulation of plants, that is by introducing into and expressing recombinant nucleic acid molecules in plants. Such approaches have the advantage of usually not being limited to one plant species but being transferable to other plant species. In EP-A 0 511 979, e.g., it was described that the expression of a prokaryotic asparagine synthetase in plant cells inter alia leads to an increased biomass production.

In WO 96/21737, e.g., the production of plants with an increased yield by the expression of deregulated or unregulated fructose-1,6-bisphosphatase due to the increase of the photosynthesis rate is described.

Nevertheless, there still is a need of generally applicable processes for improving the yield in plants interesting for agriculture or forestry.

Therefore, the problem underlying the present invention is to provide further processes for increasing the yield in plants.

This problem is solved according to the invention by providing the embodiments characterized in the claims.

Therefore, the present invention relates to a process for increasing the yield in plants, characterized in that recombinant DNA molecules stably integrated into the genome of plants are expressed, comprising

- (a) a region allowing the transcription specifically in the companion cells; and operatively linked thereto
- (b) a nucleotide sequence encoding a polypeptide selected from the group consisting of:
 - (i) proteins with an enzymatic activity that cleaves sucrose;
 - (ii) sucrose transporters;
 - (iii) proteins the activity of which leads to the stimulation of the proton gradient located at the plasma membrane of plant cells; and
 - (iv) citrate synthases (E.C. 4.1.3.7).

It was surprisingly found that the expression of the above-described proteins specifically in the phloem of plants leads to a dramatic increase in yield.

The term "increase in yield" preferably relates to an increase of the biomass production, in particular when determined as the fresh weight of the plant.

Such an increase in yield preferably refers to the so-called "sink" organs of the plant, which are the organs that take up the photoassimilates produced during photosynthesis. Particularly preferred are parts of plants which can be harvested, such as seeds, fruits, storage roots, roots, tubers, flowers, buds, shoots, stems or wood. The increase in yield according to the invention is at least 3 % with regard to the biomass in comparison to non-transformed plants of the same genotype when cultivated under the same conditions, preferably at least 10 % and particularly preferred at least 20 %.

The above-described proteins have in common that when they are expressed in the phloem their biological activity leads to an increased loading of the phloem with photoassimilates.

In the context of the present invention photoassimilates are understood to be sugars and/or amino acids.

According to the invention the nucleotide sequence mentioned in (b) can usually encode a plant protein or a bacterial protein or a protein originating from fungi or animal organisms.

In a preferred embodiment the nucleotide sequence encodes a sucrose synthase (E.C. 2.4.1.13), preferably a plant sucrose synthase, in particular from *Solanum tuberosum*, and particularly preferred the type expressed in the tubers of *S. tuberosum*. Such sequences are, for example, described in Salanoubat and Belliard (Gene 60 (1987), 47-56) and are available in the EMBL gene bank under accession number X67125.

In a further preferred embodiment the nucleotide sequence encodes a sucrose phosphorylase (E.C. 2.4.1.7).

Sequences encoding sucrose phosphorylase are, for example, known from WO 96/24679.

In another preferred embodiment the nucleotide sequence encodes an invertase (E.C. 3.2.1.26), preferably an invertase from a microorganism, in particular from a fungus of the genus *Saccharomyces*, preferably from *S. cerevisiae*. Particularly preferred are sequences encoding a cytosolic invertase (Sonnewald et al., Plant J. 1 (1991), 95-106).

According to the invention a sucrose transporter is understood to be a transporter transporting sucrose in plant systems across a membrane. Such a transporter preferably is of plant origin (for example EMBL gene bank accession number G21319). Particularly preferred the sequence described in (b) encodes a sucrose transporter from spinach (*Spinacia oleracea*), in particular with the sequence of the clone SoSUT1, as, e.g., described in Riesmeier et al. (EMBO J. 11 (1992), 4705-4713).

In a further preferred embodiment the protein that stimulates the proton gradient located at the plasma membrane is a proton ATPase.

In this case, the sequence described in (b) preferably encodes a protein from a microorganism, in particular a fungus of the genus *Saccharomyces*, preferably from *S. cerevisiae*.

In a particularly preferred embodiment the sequence encodes the proton ATPase PMA1 from *S. cerevisiae* (Serrano et al., *Nature* 319 (1986), 689-693; EMBL gene bank) or a version of this proton ATPase from *S. cerevisiae* which is truncated at the 3' end, in particular the ATPase Δ PAM1 as described in Example 3 of the present invention.

Alternatively, the nucleotide sequence can also encode a proton ATPase from plants, preferably a proton ATPase from *Solanum tuberosum*.

Particularly preferred are sequences encoding the proton ATPase PHA2 from potato (Harms et al, *Plant Mol. Biol.* 26 (1994), 979-988; EMBL gene bank X76535) or a version of this proton ATPase from potato which is truncated at the 3' end, in particular the ATPase Δ PHA2 as described in Example 4 of the present invention.

According to the invention the citrate synthase can be any citrate synthase, for example those from bacteria, fungi, animals or plants. DNA sequences encoding citrate synthase are known, for example, from the following organisms: *Bacillus subtilis* (U05256 and U05257), *E. coli* (V01501), *R. prowazekii* (M17149), *P. aeruginosa* (M29728), *A. anitratum* (M33037) (see Schendel et al., *Appl. Environ. Microbiol.* 58 (1992), 335-345 and references cited therein), *Haloferax volcanii* (James et al., *Biochem. Soc. Trans.* 20 (1992), 12), *Arabidopsis thaliana* (Z17455) (Unger et al., *Plant Mol. Biol.* 13 (1989), 411-418), *B. coagulans* (M74818), *C. burnetti* (M36338) (Heinzen et al., *Gene* 109 (1990), 63-69), *M. smegmatis* (X60513), *T. acidophilum* (X55282), *T. thermophila* (D90117), pig (M21197) (Bloxham et al., *Proc. Natl. Acad. Sci. USA* 78 (1981), 5381-5385), *N. crassa* (M84187) (Ferea et al., *Mol. Gen. Genet.* 242 (1994), 105-110), *S. cerevisiae* (Z11113, Z23259, M14686, M54982, X00782) (Suissa et al., *EMBO J.* 3 (1984), 1773-1781) and potato (EP 95 91 3066.7).

The numbers in brackets are the corresponding accession numbers in the GenEMBL data base.

The nucleotide sequences according to the invention can generally encode any appropriate proteins from any organism, in particular from plants, fungi, bacteria or animals. The sequences preferably encode proteins from plants or fungi. Preferably, the plants are higher plants, in particular starch or oil storing useful plants, for example potato or cereals such as rice, maize, wheat, barley, rye, triticale, oat, millet, etc., as well as spinach, tobacco, sugar beet, soya, cotton etc.

The fungi preferably are of the genus *Saccharomyces*, *Schizosaccharomyces*, *Aspergillus* or *Neurospora*, in particular *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus flavus*, *Aspergillus niger* or *Neurospora crassa*.

In a preferred embodiment of the process according to the invention the region mentioned in (a), which guarantees a companion cell specific transcription, is the promoter of the *rolC* gene from *Agrobacterium rhizogenes*.

This promoter is, for example, described in Schmülling et al. (*Plant Cell* (1989), 665-671) and Kühn (Characterization and localization of the sucrose carrier SUT1 in *Solanaceae*, Doctoral Thesis (1991), Freie Universität Berlin, biology department). Preferably, the region of the promoter is used that has the nucleotide sequence described in Seq ID No. 1.

Apart from the *rolC* promoter mentioned above the person skilled in the art can without further ado use other promoters for a companion cell specific expression. Further companion cell specific promoters are described in the literature, such as the promoter of the sucrose transporter from *Arabidopsis thaliana* (Truernit and Sauer, *Planta* 196 (1995), 564-570).

Furthermore, for different RNAs and proteins their specific occurrence in the companion cells has been described in the literature (see, for example, Foley et al., *Plant Mol. Biol.* 30 (1996), 687-695; DeWitt, *Plant J.* 1 (1991), 121-128; Stadler et al., *Plant Cell* 7 (1995), 1545-1554). Starting from a known protein it is possible for the person skilled in the art without further ado to isolate the cDNA by means of antibodies or by using oligonucleotides derived from the amino acid sequence (cf.,

e.g., Sambrook et al, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), Cold Spring Harbor, NY.). Starting from the cDNAs obtained this way it is furthermore possible to screen established genomic libraries from the corresponding organism and to identify genomic fragments. By comparing the nucleotide sequence of the cDNA and of the genomic clone the position of the promoter can be roughly determined. The specificity of the promoter can be verified in a transgenic situation by using chimeric genes consisting of the promoter and indicator genes, such as the β -glucuronidase (cf., e.g., Kertbundit et al., Proc. Natl. Acad. Sci. USA 88 (1991), 5212-5216).

The process according to the invention can in principle be applied to any plant. Therefore, monocotyledonous as well as dicotyledonous plant species are particularly suitable. The process is preferably used with plants that are interesting for agriculture, horticulture and/or forestry.

Examples thereof are vegetable plants such as, for example, cucumber, melon, pumpkin, egg plant, zucchini, tomato, spinach, cabbage species, peas, beans, etc., as well as fruits such as, for example, pears, apples, etc.

Furthermore, oil storing plants are suitable such as, for example, rape, sunflower, soya. In a particularly preferred embodiment starch storing plants are suitable, in particular such as cereals (rice, maize, wheat, rye, oats, triticale, millet, barley), potato, cassava, sweet potato, etc.

The process can also be applied for sucrose storing plants such as, for example, sugar beet and sugar cane, but also for other useful plants such as, for example, cotton, tobacco, types of wood, wine, hops etc.

The invention further relates to recombinant nucleic acid molecules, containing

- (a) a region allowing the transcription specifically in the companion cells of plants; and operatively linked thereto
- (b) a nucleotide sequence encoding a polypeptide, selected from the group consisting of
 - (i) sucrose synthases;
 - (ii) sucrose phosphorylases;
 - (iii) sucrose transporters;

- (iv) proteins the activity of which leads to the stimulation of the proton gradient located at the plasma membrane of plant cells; and
- (v) citrate synthases.

With regard to the preferred embodiments of such molecules, the same applies to the region mentioned in (a) and the nucleotide sequence mentioned in (b) what was already mentioned above in connection with the process of the invention.

The invention also relates to vectors containing nucleic acid molecules of the invention, in particular those which are suitable for the transformation of plant cells as well as for the integration of foreign DNA into the plant genome.

The present invention further relates to plant cells transformed with a nucleic acid molecule of the invention and containing it stably integrated into the genome. These cells differ from naturally occurring plant cells for example in that a nucleic acid molecule of the invention is integrated into the genome of the cell at a location where it does not naturally occur.

The invention further relates to transgenic plants containing plant cells of the invention and, due to the expression of the recombinant nucleic acid molecule integrated into the genome in the companion cells of the plants, showing an increased yield in comparison with corresponding non-transformed plants that were cultivated under the same conditions.

The present invention further relates to propagation material of plants of the invention containing the above-described plant cells of the invention. The term "propagation material" in particular comprises seeds, fruits, tubers, rhizomes, cuttings, calli, cell cultures, etc.

Finally, the present invention relates to the use of recombinant nucleic acid molecules containing a region allowing the transcription specifically in the companion cells of plants and, operatively linked thereto, a nucleotide sequence encoding a polypeptide selected from the group consisting of:

- (i) proteins with sucrose cleaving enzymatic activity;
- (ii) sucrose transporters;
- (iii) proteins the activity of which leads to the stimulation of the proton gradient located at the plasma membrane; and
- (iv) citrate synthases

for the expression in transgenic plants for increasing the yield.

The encoded proteins preferably are the proteins further described above.

Processes for the transformation of monocotyledonous and dicotyledonous plants are known to the person skilled in the art.

For the introduction of DNA into a plant host cell a variety of techniques is available. These techniques comprise the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation means, the fusion of protoplasts, the injection, the electroporation of DNA, the introduction of DNA by means of the biolistic method as well as further possibilities.

For the injection and electroporation of DNA in plant cells the plasmids do not have to fulfill specific requirements. Simple plasmids such as pUC derivatives can be used.

The use of agrobacteria for the transformation of plant cells has extensively been examined and sufficiently disclosed in the specification of EP-A 120 516, in Hoekema (In: The Binary Plant Vector System Offsetdrukkerij Kanters B.V., Alblasterdam (1985), Chapter V), Fraley et al. (Crit. Rev. Plant. Sci. 4, 1-46) and An et al. (EMBO J. 4 (1985), 277-287).

For the transfer of the DNA to the plant cell plant explants can be co-cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. From the infected plant material (for example leaf explants, segments of stems, roots but also protoplasts or suspension cultivated plant cells) whole plants can be regenerated in a suitable medium which may contain antibiotics or biozides for the selection of transformed cells. The plants obtained that way can then be examined for the presence of the introduced DNA. Other possibilities for the introduction of foreign DNA using the biolistic method or by protoplast transformation are known (cf., e.g., Willmitzer, L., 1993 Transgenic plants. In: Biotechnology, A Multi-Volume Comprehensive Treatise (H.J. Rehm, G. Reed, A. Pühler, P. Stadler, eds.), Vol. 2, 627-659, VCH Weinheim-New York-Basel-Cambridge).

The transformation of dicotyledonous plants via Ti-plasmid-vector systems with the help of *Agrobacterium tumefaciens* is well-established. Recent studies have indicated that also monocotyledonous plants can be transformed by means of vectors based on *Agrobacterium* (Chan et al., *Plant Mol. Biol.* 22 (1993), 491-506; Hiei et al., *Plant J.* 6 (1994), 271-282; Deng et al., *Science in China* 33 (1990), 28-34; Wilmink et al., *Plant Cell Reports* 11 (1992), 76-80; May et al., *Bio/Technology* 13 (1995), 486-492; Conner and Domisse; *Int. J. Plant Sci.* 153 (1992), 550-555; Ritchie et al., *Transgenic Res.* 2 (1993), 252-265).

Alternative systems for the transformation of monocotyledonous plants are the transformation by means of the biolistic method (Wan and Lemaux, *Plant Physiol.* 104 (1994), 37-48; Vasil et al., *Bio/Technology* 11 (1993), 1553-1558; Ritala et al., *Plant Mol. Biol.* 24 (1994), 317-325; Spencer et al., *Theor. Appl. Genet.* 79 (1990), 625-631), the protoplast transformation, the electroporation of partially permeabilized cells, as well as the introduction of DNA by means of glass fibers.

In particular the transformation of maize is described in the literature several times (cf., e.g., WO95/06128, EP 0 513 849; EP 0 465 875; Fromm et al., *Biotechnology* 8 (1990), 833-844; Gordon-Kamm et al., *Plant Cell* 2 (1990), 603-618; Koziel et al., *Biotechnology* 11 (1993), 194-200). In EP 292 435 and in Shillito et al. (*Bio/Technology* 7 (1989), 581) a process is described with the help of which and starting from a mucus-free, soft (friable) maize callus fertile plants can be obtained. Prioli and Söndahl (*Bio/Technology* 7 (1989), 589) describe the regenerating and obtaining of fertile plants from maize protoplasts of the Cateto maize inbred line Cat 100-1.

The successful transformation of other cereal species has also been described, for example for barley (Wan and Lemaux, see above; Ritala et al., see above) and for wheat (Nehra et al., *Plant J.* 5 (1994), 285-297).

Once the introduced DNA has been integrated into the genome of the plant cell, it usually is stable there and is also contained in the progenies of the originally transformed cell. It usually contains a selection marker which makes the transformed plant cells resistant to a biozide or an antibiotic such as kanamycin, G 418, bleomycin, hygromycin or phosphinotricin and others. Therefore, the individually chosen marker should allow the selection of transformed cells from cells lacking the introduced DNA.

The transformed cells grow within the plant in the usual way (see also McCormick et al., Plant Cell Reports 5 (1986), 81-84). The resulting plants can be cultured normally. Seeds can be obtained from the plants.

Two or more generations should be cultivated to make sure that the phenotypic feature is maintained stably and is transmitted. Seeds should be harvested to make sure that the corresponding phenotype or other properties are maintained.

Figure 1 schematically shows the construction of the plasmid pBinRoIC-SS.

Figure 2a shows the analysis of the sucrose synthase (SS) activity in leaves of transgenic potato plants which had been transformed with the RoIC-SS construct. The enzyme activity was determined according to Zrenner et al. (Plant J. 7 (1995), 97-107). The activity is indicated in $\mu\text{mol hexose equivalents}/(\text{min} \times \text{g fresh weight})$.

The columns represent the average values of three samples per genotype. The standard deviation is also indicated.

Figure 2b shows the analysis of the tuber yield of transgenic potato plants which had been transformed with the RoIC-SS construct. The columns represent average values of ten to fifteen plants per genotype. The standard deviation is also indicated. The tuber yield is indicated in g per fresh weight.

Figure 2c shows the analysis of the tuber starch of transgenic potato plants that had been transformed with the RoIC-SS construct. For this purpose tubers harvested from ten to fifteen plants per genotype were collected and the starch content of the tubers was determined according to Von Schéele et al. (Landw. Vers. Sta. 127 (1937), 67-96).

Figure 3 schematically shows the construction of the plasmid pBinRoIC-Suc2.

Figure 4 schematically shows the construction of the plasmid pBinRoIC- ΔPMA1 .

Figure 5 schematically shows the cloning strategy of Δ PMA1.

Step from A to B:

The H^+ -ATPase Δ PMA1, which was truncated at the 3' end, was amplified via PCR with the PMA1 cDNA as the matrix and complementary internal primers (A). The flanking cleavage sites of the PCR product (B) were introduced via the correspondingly synthesized primers.

Step from B to C:

PstI/NotI digestion and cloning of the PCR fragment into the *E. coli* vector SK- via PstI/NotI cleavage sites (C).

Step from C to D:

BclI/SpeI digestion of the plasmid SK- Δ PMA1 and cloning of the fragment into the compatible BamHI/XbaI cleavage sites of pBinRoIC (D)

Figure 6 shows the results of the polymerase chain reaction with specific oligonucleotides indicating the stable integration of Δ PMA1 in the genome of transgenic plants which had been obtained by transformation with the roIC- Δ PMA2 construct. Size of the PCR product = 730 bp; WT = wildtype; M = marker.

Figure 7 schematically shows the construction of the plasmid pBinRoIC- Δ PHA2.

Figure 8 schematically shows the cloning strategy of Δ PHA2.

Step from A to B:

The H^+ -ATPase Δ PHA2, which was truncated at the 3' end, was amplified via PCR with the PHA2 cDNA as the matrix and complementary internal primers (A). The flanking cleavage sites of the PCR product (B) were introduced via the correspondingly synthesized primers.

Step from B to C:

PstI/EcoRI digestion and cloning of the PCR fragment into the *E. coli* vector SK- via PstI/EcoRI cleavage sites (C).

Step from C to D:

BglII/SpeI digestion of the plasmid SK- Δ PHA2 and cloning of the fragment into the compatible BamHI/XbaI cleavage sites of pBinRolC (D)

Figure 9 shows the results of the polymerase chain reaction with specific oligonucleotides indicating the stable integration of Δ PHA2 in the genome of transgenic plants which had been obtained by transformation with the rolC- Δ PHA2 construct. Size of the PCR product = 758 bp; WT = wildtype; M = marker.

Figure 10 schematically shows the construction of the plasmid pBinRolC-SoSUT1.

Figure 11 schematically shows the construction of the plasmid pBinRolC-CiSy.

Figure 12 shows the results of the determination of the sucrose content in parenchymatic samples of tubers of engrafted potato plants enriched with vascular tissue. The genotypes used for engrafting are the lines RolC-Suc2-#25 (cytosolic invertase) and wildtype *Solanum tuberosum* var. Désirée. The sucrose content was determined according to Stitt et al. (Methods Enzymol. 174 (1989), 518-522). The columns represent the average values of 12 samples per engrafted type. The standard deviation is indicated. The sucrose content is indicated as μ mol hexose equivalents/g fresh weight.

Figure 13 shows the analysis of phloem exudates of Δ PMA1 leaves which were kept under light for six hours in a $^{14}\text{CO}_2$ atmosphere. The sucrose content was determined according to Stitt et al. (loc. cit). The columns represent the average values of four to five samples per genotype. The standard deviation is indicated.

Figure 14 shows the tuber yield (in gram fresh weight) of Δ PMA1 plants. The columns represent the average values of six plants per genotype. The standard deviation is indicated. The tuber yield is indicated in g fresh weight.

Figure 15 shows the tuber yield (in gram fresh weight) of Δ PHA2 plants. The columns represent average values of four to five plants per genotype. The standard deviation is indicated. The tuber yield is indicated in g fresh weight.

The following examples illustrate the invention.

Example 1

Production of the plasmid pBinRoIC-SS and production of transgenic potato plants

The plasmid pBinRoIC-SS contains the three fragments A, B and C in the binary vector pBin19 (Bevan, Nucl. Acids Res. 12 (1984), 8711) (cf. Fig.1).

The fragment A comprises the *rolC* promoter from *Agrobacterium rhizogenes*. The *rolC* promoter contains as an *EcoRI*/*Asp718* DNA fragment of 1138 bp (Lerchl et al., Plant Cell 7 (1995), 259-270) the DNA region (position: 11306 to position 12432) of the TL-DNA of the Ri-agropin-type plasmid from *A. rhizogenes* (Slightom et al., J. Biol. Chem. 261 (1986), 108-121). The fragment A is inserted into the *EcoRI* and *Asp718* cleavage sites of the polylinker of pBin19.

The fragment B contains the coding region (position: 76 to position 2493) of the cDNA of the sucrose synthase (SS) from *Solanum tuberosum* (Salanoubat and Belliard, Gene 60 (1987), 47-56). The fragment B was obtained as *Bam*HI fragment of 2427 bp from the vector pBluescript SK⁺, in which it is inserted into the *Bam*HI cleavage site of the polylinker. The fragment B was inserted in sense orientation in the vector pBin19 into the *Bam*HI cleavage site, that is downstream of the *rolC* promoter in an orientation allowing the transcription of a translatable RNA.

The fragment C contains the polyadenylation signal of the Gene 3 of the T-DNA of the Ti plasmid pTi ACH 5 (Gielen et al., EMBO J. 3 (1984), 835-846), in particular the nucleotides 11749-11939, which was isolated as a PvuII/HindIII fragment from the plasmid pAGV 40 (Herrera-Estrella et al., Nature 303 (1983), 209-213) and which upon addition of SphI linkers was cloned into the PvuII cleavage site between the SphI and the HindIII cleavage site of the polylinker of pBin19.

The resulting plasmid pBinRoIC-SS was introduced into potato plant cells via the gene transfer mediated by *Agrobacterium tumefaciens*. For this purpose ten small leaves of a potato sterile culture (*Solanum tuberosum* L. cv. Désirée) wounded with the scalpel were put into 10 ml MS medium (Murashige and Skoog, Physiol. Plant. 15 (1962)), 473 with 2% of sucrose containing 50 µl of a *Agrobacterium tumefaciens* overnight culture grown under selection. After 3 to 5 minutes of gentle shaking a further incubation followed for two days in the dark. Then the leaves were put on MS medium with 1.6 % glucose, 5 mg/l naphthyl acetic acid, 0.2 mg/l benzylaminopurin, 250 mg/l claforan, 50 mg/l kanamycin and 0.8 % bacto-agar for callus induction. After an incubation of one week at 25 °C and 3000 lux the leaves were put on MS medium with 1.6 % glucose, 1.4 mg/l zeatin ribose, 20 µg/l naphthyl acetic acid, 20 µg/l giberellic acid, 250 mg/l claforan, 50 mg/l kanamycin and 0.8 % bacto-agar.

The analysis of the leaves of a number of plants transformed with this vector system unambiguously indicated the presence of an increased sucrose synthase activity. This is a result of the expression of the sucrose synthase gene from potato contained in pBinRoIC-SS (cf. Figure 2a).

The analysis of the tuber yield (tuber fresh weight in gram) of plants transformed with this vector system and showing an increased sucrose synthase activity unambiguously showed an increased tuber yield. This is also a result of the expression of the sucrose synthase gene from potato contained in pBinRoIC-SS (cf. Figure 2b).

The starch content of potato tubers is linearly dependent on the density of the tubers (von Schéele et al., Landw. Vers. Sta. 127 (1937), 67-96). The analysis of the density of transgenic tubers of plants which had been transformed with the vector system pBinRoIC-SS having an increased sucrose synthase activity surprisingly showed an

increased starch content. This is a result of the expression of the sucrose synthase gene from potato contained in pBinRoIC-SS (cf. Figure 2c).

Example 2

Production of the plasmid pBinRoIC-Suc2 and production of transgenic potato plants

The plasmid pBinRoIC-Suc2 contains the three fragments A, B and C in the binary vector pBin19 (Bevan, loc. cit.) and is illustrated in Figure 3.

The fragments A and C correspond to the fragments A and C as described in Example 1.

The fragment B contains the coding region (position: 845 to position: 2384) of the gene of the cytosolic invertase from yeast (*Saccharomyces cerevisiae*). The fragment B was obtained as a BamHI fragment with a length of 1548 bp from the vector pBluescript SK⁻ in which it is inserted in the BamHI cleavage site of the polylinker. The fragment B is inserted in sense orientation into pBin19 in the BamHI cleavage site.

The plasmid pBinRoIC-Suc2 was introduced into potato plant cells via gene transfer mediated by *Agrobacterium*. From transformed cells whole plants were regenerated. Such plants show in comparison to non-transformed plants an increased yield (increased biomass).

Example 3

Production of the plasmid pBinRoIC- Δ PMA1 and production of transgenic potato plants

The plasmid pBinRoIC- Δ PMA1 contains the three fragments A, B and C in the binary vector pBin19 (Bevan, loc. cit.) and is schematically illustrated in Figure 4.

The fragments A and C correspond to the fragments A and C as described in Example 1.

The fragment B contains the coding region (position: 937 to position: 3666) of the gene of the proton ATPase PMA1 from the yeast *Saccharomyces cerevisiae* (Serrano et al., Nature 319 (1986), 689-693). The fragment B was obtained by means of polymerase chain reaction (PCR). For this purpose the 3' end of the coding region of the gene PMA1 was truncated on purpose by 27 bp and at the same time a necessary new stop codon was introduced. The DNA fragment modified this way was called Δ PMA1. The fragment B was inserted, as a BclI/SpeI fragment with a length of 2739 bp, in sense orientation into the BamHI (compatible insertion site for BclI restriction sites) and XbaI (compatible insertion site for SpeI restriction sites) cleavage sites of the vector pBin19.

The fragment B was obtained as BclI/SpeI fragment from the vector pBluescript SK⁻ in which it is inserted via the cleavage sites NotI and PstI of the polylinker (cf. Fig. 5). The plasmid pBinRoIC- Δ PMA1 was introduced into potato plant cells via the gene transfer mediated by *Agrobacterium*. Whole plants were regenerated from transformed cells.

The stable integration of Δ PMA1 in the genome of transgenic plants which had been obtained by using the vector system pBinRoIC- Δ PMA1 was detected by means of polymerase chain reaction (PCR) (cf. Fig. 6).

The transformed plants show an increased yield (increased biomass) in comparison to non-transformed plants (see Figures 13 and 14).

Example 4

Production of the plasmid pBinRoIC- Δ PHA2 and production of transgenic potato plants

The plasmid pBinRoIC- Δ PHA2 contains the three fragments A, B and C in the binary vector pBin19 (Bevan, loc. cit.) and is schematically illustrated in Figure 7.

The fragments A and C correspond to the fragments A and C as described in Example 1.

The fragment B contains the coding region (position: 64 to position: 2672) of the cDNA of the proton-ATPase PHA2 (Harms et al., Plant Mol. Biol. 26 (1994), 979-988). The fragment B was obtained by means of polymerase chain reaction (PCR). For this purpose the 3' end of the coding region of the gene PHA2 was on purpose truncated by 249 bp, and at the same time two new stop codons were introduced. The DNA fragment modified that way was called Δ PHA2. The fragment B was inserted in sense orientation as a BglII/SpeI fragment with a length of 2631 bp into the BamHI (compatible insertion site for BglII restriction sites) and XbaI (compatible insertion site for SpeI restriction sites) cleavage sites of the vector pBin19.

The fragment B was obtained as BglII/SpeI fragment from the vector pBluescript SK⁺, in which it is inserted into the EcoRI and PstI cleavage sites of the polylinker sequence (cf. Fig. 8: cloning strategy Δ PHA2).

The plasmid pBinRoIC- Δ PHA2 was introduced into potato plant cells via the gene transfer mediated by *Agrobacterium*. Whole plants were regenerated from transformed cells.

The stable integration of Δ PHA2 in the genome of transgenic plants which had been obtained using the vector system pBinRoIC- Δ PHA2 was detected by means of polymerase chain reaction (PCR) (cf. Fig. 9).

The transformed plants show an increased yield (increased biomass) in comparison to non-transformed plants (see Figure 15).

Example 5

Production of the plasmid pBinRoIC-SoSUT1 and production of transgenic potato plants

The plasmid pBinRoIC-SoSUT1 contains the three fragments A, B and C in the binary vector pBin19 (Bevan, loc. cit.) and is schematically illustrated in Figure 10.

The fragments A and C correspond to the fragments A and C as described in Example 1.

The fragment B contains the cDNA (position: 1 to position: 1969) encoding a sucrose transporter from spinach (*Spinacia oleracea*) (Riesmeier et al., EMBO J. 11 (1992), 4705-4713; accession number X67125 and S51273). The fragment B was obtained as a NotI fragment from the vector pBluescript SK⁺, in which it is inserted via a NotI linker sequence. For the cloning into the SmaI cleavage site of the vector pBin19 the sticky ends of the fragment resulting from the NotI digestion were converted to blunt ends and inserted in sense orientation into the pBin19. The resulting plasmid was called pBinRoIC-SoSUT1.

It was introduced into potato plant cells via the gene transfer mediated by *Agrobacterium*. Whole plants were regenerated from transformed cells.

Plants transformed that way show an increased yield (increased biomass) in comparison to non-transformed plants.

Example 6

Production of the plasmid pBinRoIC-CiSy and production of transgenic potato plants

The plasmid pBinRoIC-CiSy contains the three fragments A, B and C in the binary vector pBin19 (Bevan, Nucl. Acids Res. 12 (1984), 8711) modified according to Becker (Nucl. Acids Res. 18 (1990), 203) (cf. Fig. 11).

The fragment A comprises the *rolC* promoter from *Agrobacterium rhizogenes*. The *rolC* promoter contains as an EcoRI/Asp718 DNA fragment with a length of 1143 bp (Lerchl et al., The Plant Cell 7 (1995), 259-270) the DNA region (position: 11306 to position 12432) of the TL-DNA of the Ri-agropin type plasmid from *A. rhizogenes* (Slightom et al., J. Biol. Chem. 261 (1986), 108-121). The fragment A is inserted in the EcoRI and Asp718 cleavage sites of the polylinker of pBin19.

The fragment B contains the coding region of the cDNA of the citrate synthase (CiSy) from the fission yeast *Saccharomyces cerevisiae*. The fragment B was obtained as a BamHI fragment with a length of 1400 bp from the vector pBluescript SK⁺, in which it is inserted in the BamHI cleavage site of the polylinker (Landschütze, Studies on the influence of the acetyl-CoA synthesis and use in transgenic plants, Doctoral Thesis, Freie Universität Berlin, (1985) D83/FB15 No. 028).

The fragment C contains the polyadenylation signal of the gene 3 of the T-DNA of the Ti plasmid pTiACH 5 (Gielen et al., EMBO J. 3 (1984), 835-846), nucleotides 11749-11939, which had been isolated as a PvuII/HindIII fragment from the plasmid pAGV 40 (Herrera-Estrella et al., Nature 303 (1983), 209-213) and which after addition of SphI linkers to the PvuII cleavage site had been cloned between the SphI and HindIII cleavage site of the polylinker of pBin19.

The plasmid pBinRolC-CiSy has a length of about 13 kb.

The plasmid pBinRolC-CiSy was inserted into potato plants via the gene transfer mediated by *Agrobacterium tumefaciens*. Whole plants were regenerated from transformed cells.

The analysis of a number of plants transformed with this vector system unambiguously showed an increased biomass, which is a result of the expression of the CiSy cDNA from yeast contained in pBinRolC-CiSy.

Example 7

Grafting experiment

For grafting the shoot of a receiver plant is replaced with the shoot of a donor plant.

In this experiment the shoot of a transgenic plant (RolC-Suc2 #25) is grafted onto the stock of a wildtype plant (*Solanum tuberosum*, var. Désirée). In a control experiment a wildtype shoot is grafted onto a wildtype stock in order to rule out culturing differences in the experiments (autografting). The aim of the experiment is to examine the exclusive impact of the photosynthetic activity and photoassimilate distribution of a transgenic shoot on organs (in this case tubers) of a wildtype stock.

Potato plants were transferred from a tissue culture to soil and placed into a greenhouse. After approx. five weeks (the plants have not yet induced tuber production at this stage) the plants are grafted. For this purpose the shoot of the receiver plant which is not needed is cut off, and a wedge is cut into the stem of the receiver plant. The donor shoot to be grafted is cut at the stem end in the appropriate way and is inserted into the wedge of the receiver plant. The grafting site is fixed with an adhesive tape.

Then the grafted potato plants are kept under increased air humidity and in shadow for approx. one week. Within seven to ten days they are step by step adapted to normal greenhouse conditions. At this stage the plants are seven weeks old.

All leaves of the receiver plant are now removed, and the stem is covered from light with an aluminum sheet in order to guarantee that exclusively the photosynthetic activity and the photoassimilate distribution of the donor shoot nourishes the stock of the grafted plant.

The plants are kept in the greenhouse until the potato tubers are harvested approx. two months after the grafting and approx. three months after the planting into soil. The results of such a grafting experiment are illustrated in Figure 12.

Sequence Listing

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Max-Planck-Gesellschaft zur Foerderung der
Wissenschaften e.V
- (B) STREET: none
- (C) CITY: Berlin
- (E) COUNTRY: Germany
- (F) POSTAL CODE: none

(ii) TITLE OF INVENTION: Process for increasing the yield in plants

(iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1138 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Agrobacterium rhizogenes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```

AATTCGATAC GAAAAAGGCA AGTGCCAGGG CCATTAAAA TACGGCGTCG GAAACTGGCG      60
CCAATCAGAC ACAGTCTCTG GTCGGGAAAG CCAGAGGTAG TTTGGCAACA ATCACATCAA      120
GATCGATGCG CAAGACACGG GAGGCCTTAA AATCTGGATC AAGCGAAAAA ACTGCATGCG      180
TGATCGTTCA TGGGTTTATA GTACTGGGTT TGCTTTTCTT TGTCGTGTTG TTTGGCCTTA      240
GCCAAAGGAT GTCAAAAAAG GATGCCCATTA ATTGGGAGGA GTGGGGTAAA GCTTAAAGTT      300
GGCCCCGCTAT TGGATTTTCG GAAAGCGGCA TTGGCAAACG TGAAGATTGC TGCATTCAAG      360
ATACTTTTTC TATTTTCTGG TTAAGATGTA AAGTATTGCC ACAATCATAT TAATTACTAA      420
CATGTATATAT GTAATATAGT GCGGAAATTA TCTATGCCAA AATGATGTAT TAATAATAGC      480

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AATAATAATA TGTGTTAATC TTTTCAATC GGAATACGT TTAAGCGATT ATCGTGTGA	540
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TTTACTAG TTTAGTGGGT GCAAACTAT TATCTCTGTT TCTGAGTTA ATAAAAATA	660
AATAAGCAGG GCGAATAGCA GTTAGCCTAA GAAGGAATGG TGGCCATGTA CGTGCTTTTA	720
AGAGACCCTA TAATAAATTG CCAGCTGTGT TGCTTTGGTG CCGACAGGCC TAACGTGGGG	780
TTTAGCTTGA CAAAGTAGCG CCTTCCGCA GCATAATAA AGGTAGGCGG GTGCGTCCCA	840
TTATTAAAGG AAAAGCAAA AGCTGAGATT CCATAGACCA CAAACCACCA TTATTGGAGG	900
ACAGAACCTA TTCCCTCAGG TGGGTCGCTA GCTTTAAACC TAATAAGTAA AAACAATTAA	960
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CATCCATTAA TTAATAAATT TGTGGACCTA TACCTAATC AAATATTTT ATTATTTGCT	1080
CCAATACGCT AAGAGCTCTG GATTATAAAT AGTTTGGATG CTTCGAGTTA TGGGGTAC	1138

PCT/EP98/01878
Max-Planck-Gesellschaft et al.
Our Ref.: C 1991 PCT

Claims

1. A process for increasing the yield of plants, characterized in that recombinant DNA molecules containing
 - (a) a region allowing the transcription specifically in the companion cells; and operatively linked thereto
 - (b) a nucleotide sequence encoding a polypeptide selected from the group consisting of:
 - (i) proteins with an enzymatic activity that cleaves sucrose;
 - (ii) sucrose transporters;
 - (iii) proteins the activity of which leads to the stimulation of the proton gradients located at the plasma membrane of plant cells; and
 - (iv) citrate synthases;and which are stably integrated into the genome of plants are expressed.
2. The process of claim 1, wherein the nucleotide sequence encodes a plant protein.
3. The process of claim 1, wherein the nucleotide sequence encodes a protein from a bacterium or a fungus.
4. The process of claim 1, wherein the nucleotide sequence encodes a protein with an enzymatic activity that cleaves sucrose, selected from the group consisting of sucrose synthases, sucrose phosphorylases and invertases.
5. The process of claim 1, wherein the nucleotide sequence encodes a sucrose transporter from *Spinacia oleracea*.
6. The process of claim 1, wherein the nucleotide sequence encodes a proton ATPase.

7. The process of claim 6, wherein the nucleotide sequence encodes a proton ATPase from *Solanum tuberosum* or from *Saccharomyces cerevisiae*.
8. The process of any one of claims 1 to 7, wherein the region mentioned in (a) is the *rolC* promoter from *Agrobacterium rhizogenes*.
9. A recombinant nucleic acid molecule containing
 - (a) a region allowing the transcription specifically in the companion cells of plants; and operatively linked thereto
 - (b) a nucleotide sequence encoding a polypeptide, selected from the group consisting of
 - (i) sucrose synthases;
 - (ii) sucrose phosphorylases;
 - (iii) sucrose transporters;
 - (iv) proteins the activity of which leads to the stimulation of the proton gradient located at the plasma membrane of plant cells; and
 - (v) citrate synthases,
 wherein said recombinant nucleic acid molecule, when stably integrated into the genome of plants and expressed, leads to an increase of the yields of plants.
10. A vector containing a recombinant nucleic acid molecule of claim 9.
11. The vector of claim 10 which is suitable for the transformation of plant cells and for integration of foreign DNA into the plant genome.
12. A plant cell transformed with and containing a recombinant nucleic acid molecule of claim 9.
13. A plant containing plant cells of claim 12, wherein the plant shows an increased yield in comparison to a corresponding non-transformed plant due to the expression of the recombinant nucleic acid molecule in the companion cells of the plant.

14. Propagation material of a plant of claim 13, wherein said propagation material contains plant cells of claim 12.
15. Use of a recombinant nucleic acid molecule containing a region allowing the transcription specifically in the companion cells of plants and operatively linked thereto a nucleotide sequence encoding a polypeptide selected from the group consisting of
 - (i) proteins with an enzymatic activity that cleaves sucrose;
 - (ii) sucrose transporters;
 - (iii) proteins the activity of which leads to the stimulation of the proton gradient located at the plasma membrane; and
 - (iv) citrate synthases,for the expression in transgenic plants for increasing the yield.

Abstract

Process for increasing the yield in plants

Described are processes for increasing the yield in plants in which nucleotide sequences are expressed under the control of companion cell specific promoters. The nucleotide sequences encode proteins the expression of which leads to a stimulation of the loading of the phloem with photoassimilates.

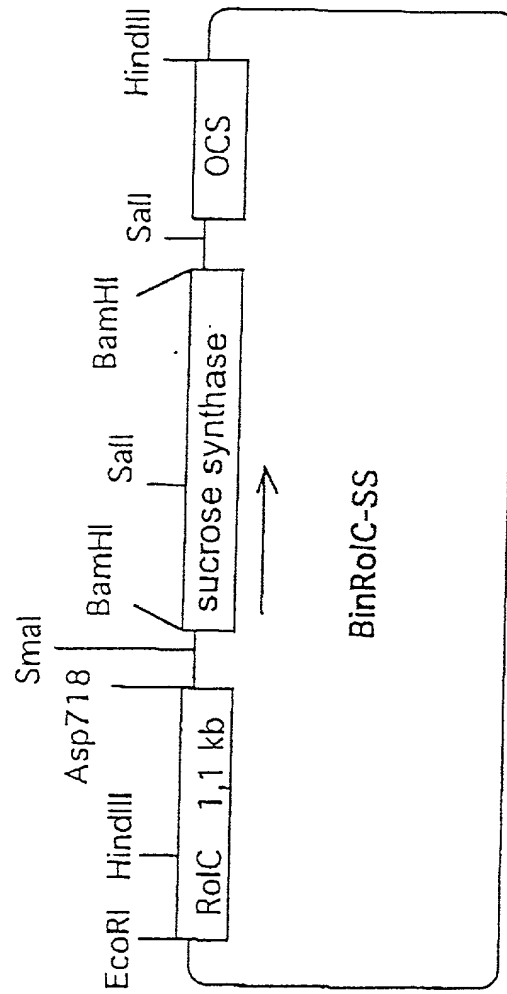


Figure 1

2/17

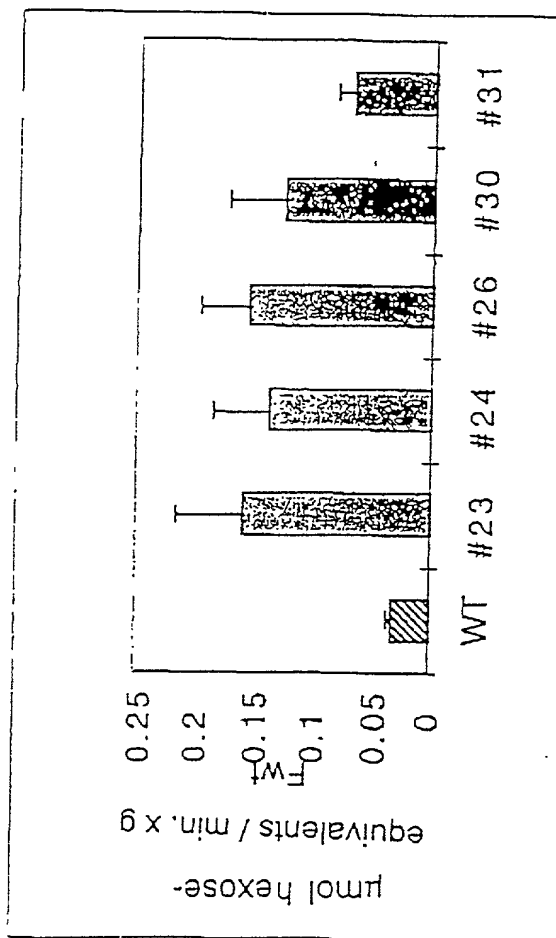


Figure 2a

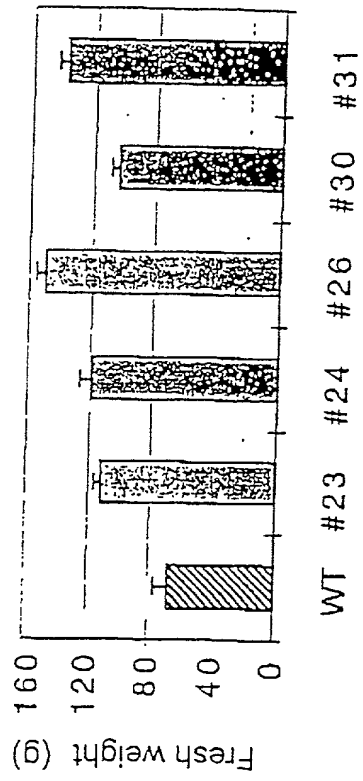


Figure 2b

4/17

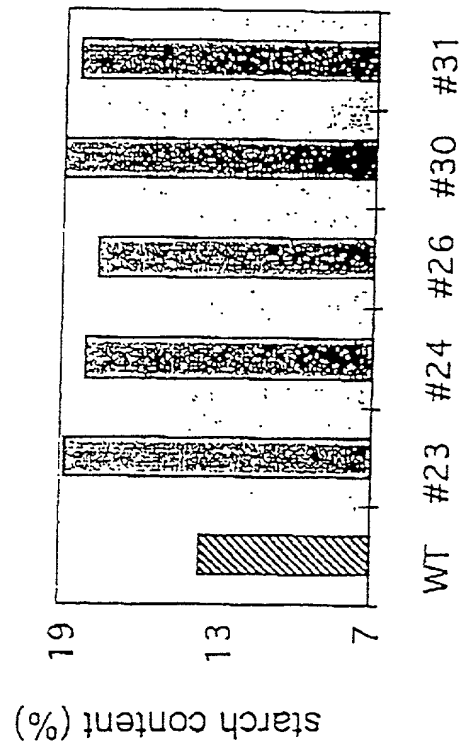


Figure 2c

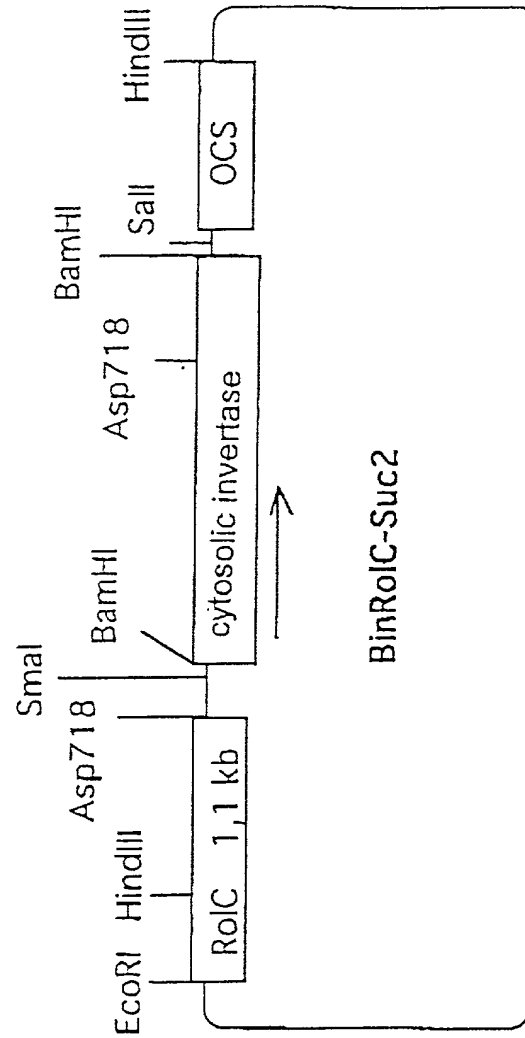


Figure 3

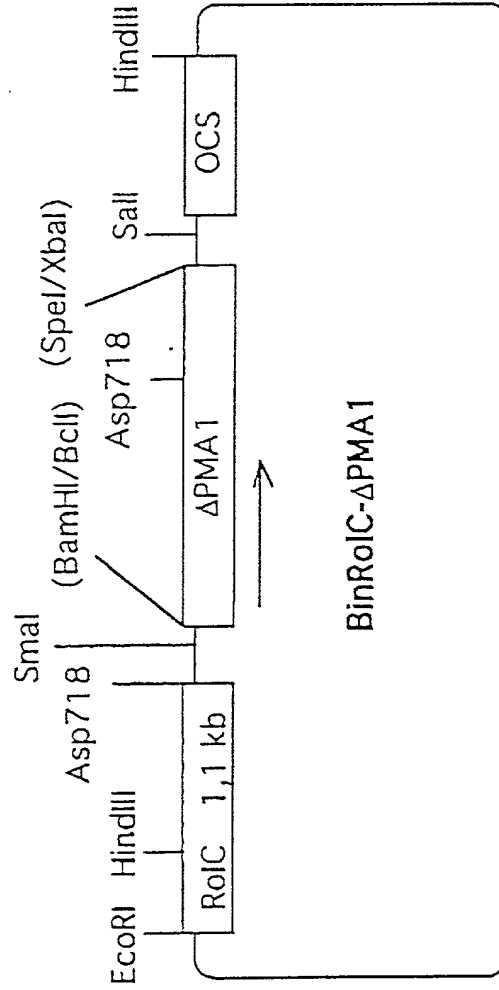


Figure 4

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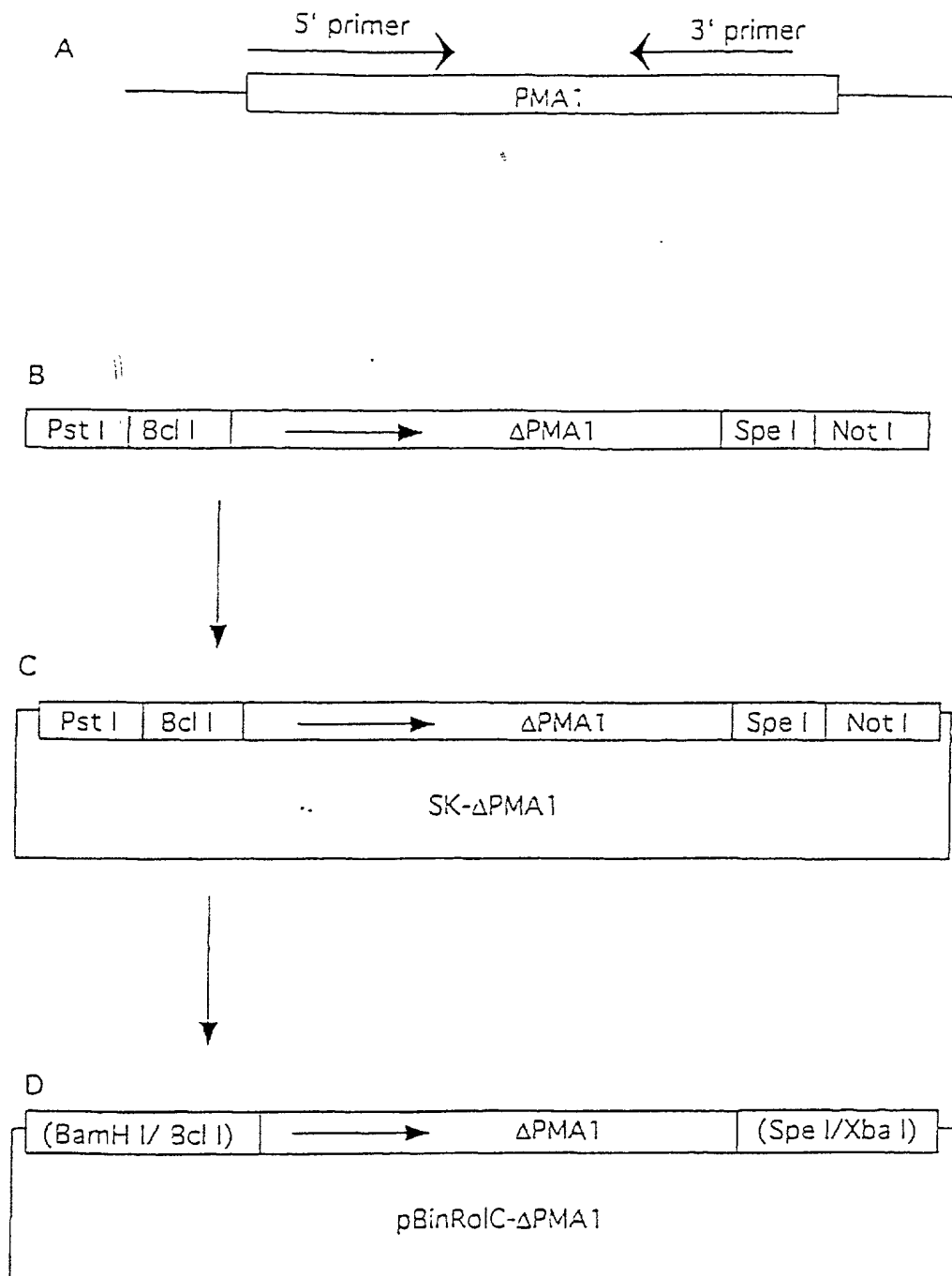


Figure 5

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M #5 #7 #18 #19 #20 WT

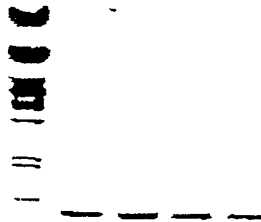


Figure 6

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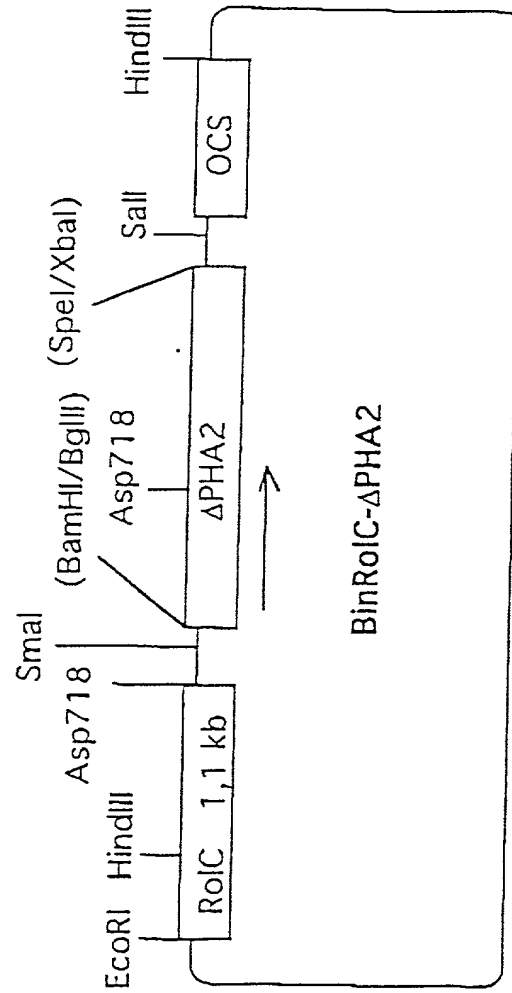


Figure 7

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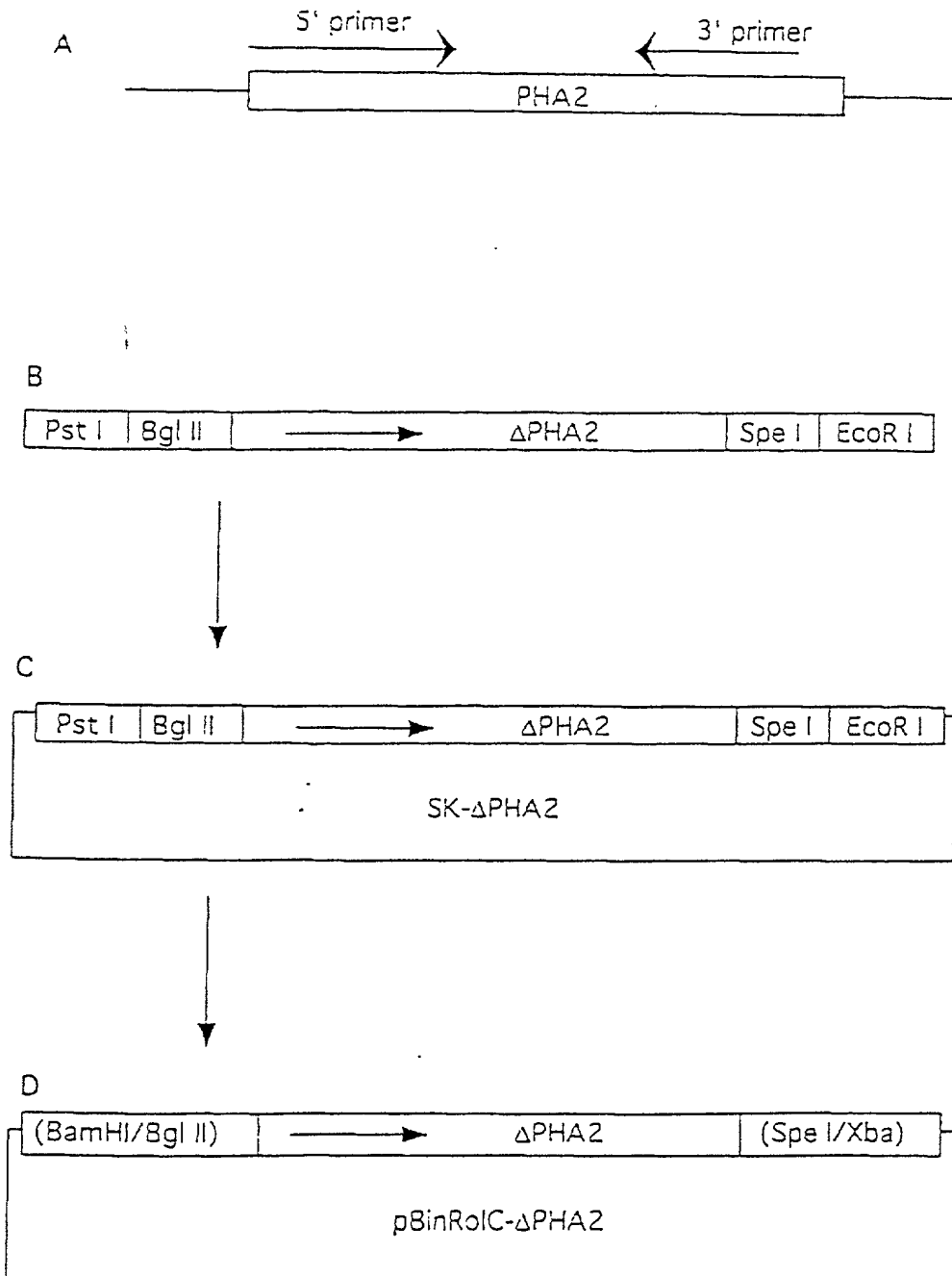


Figure 8

12/17

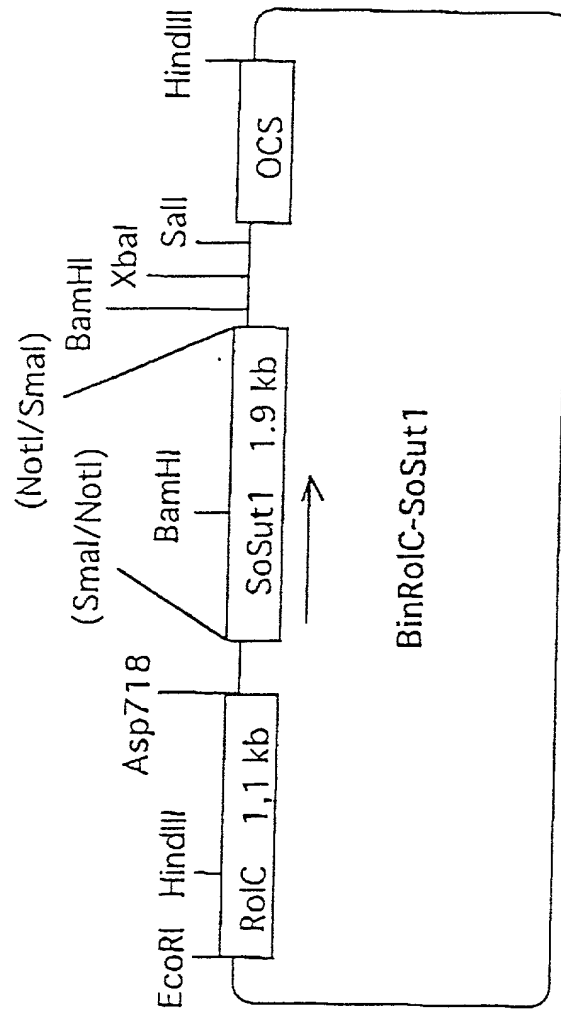


Figure 10

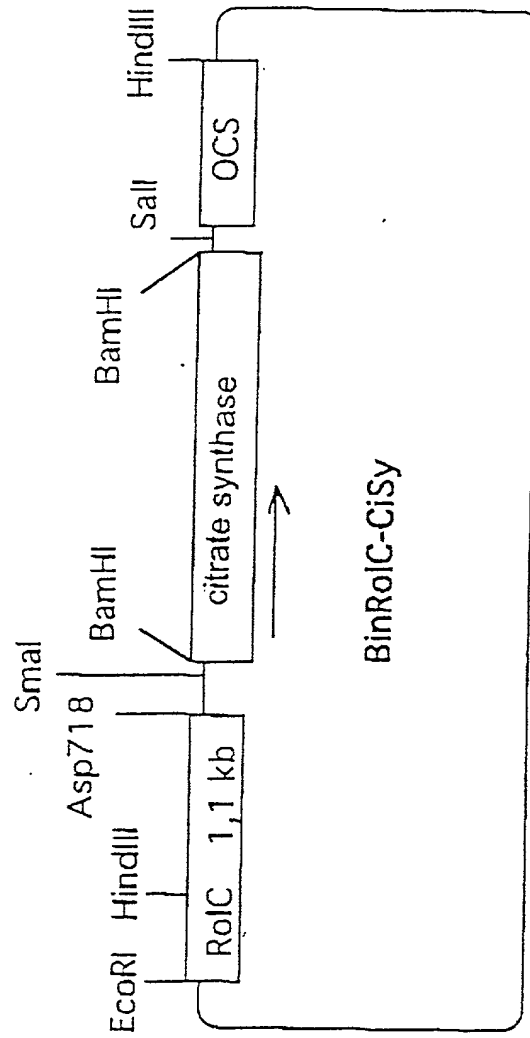


Figure 11

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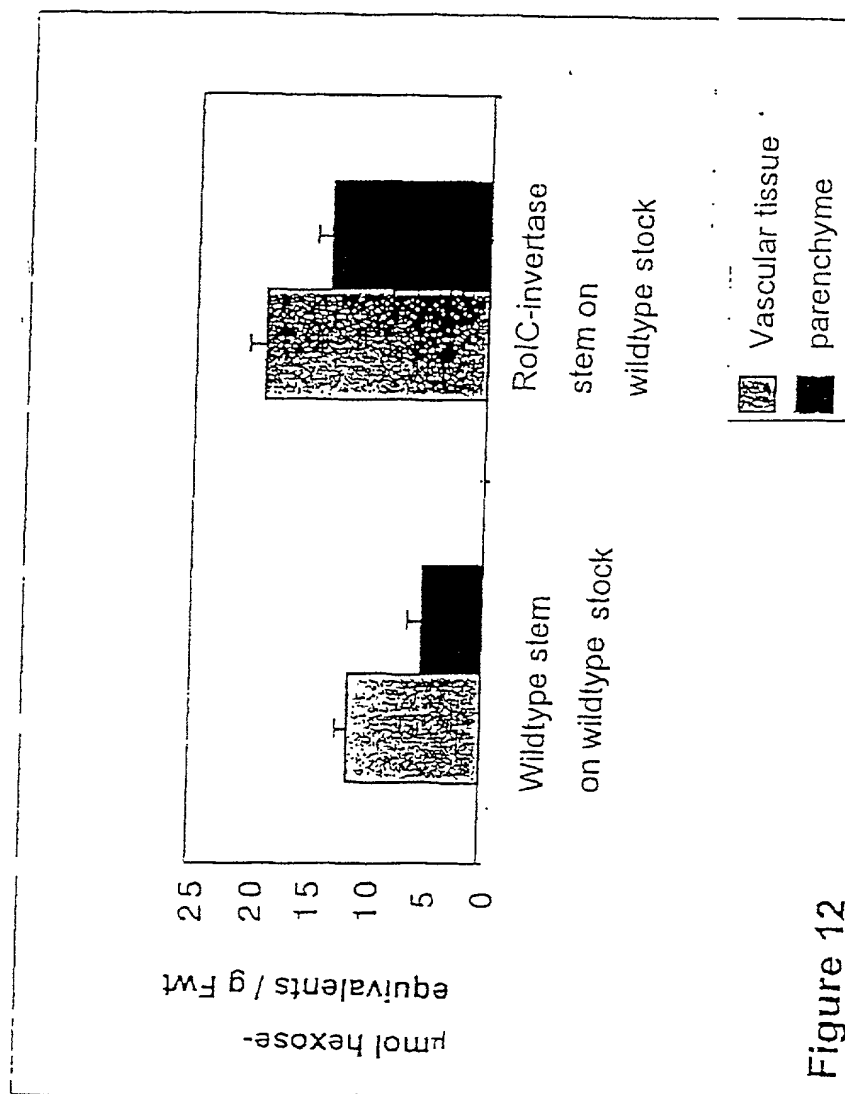


Figure 12

15/17

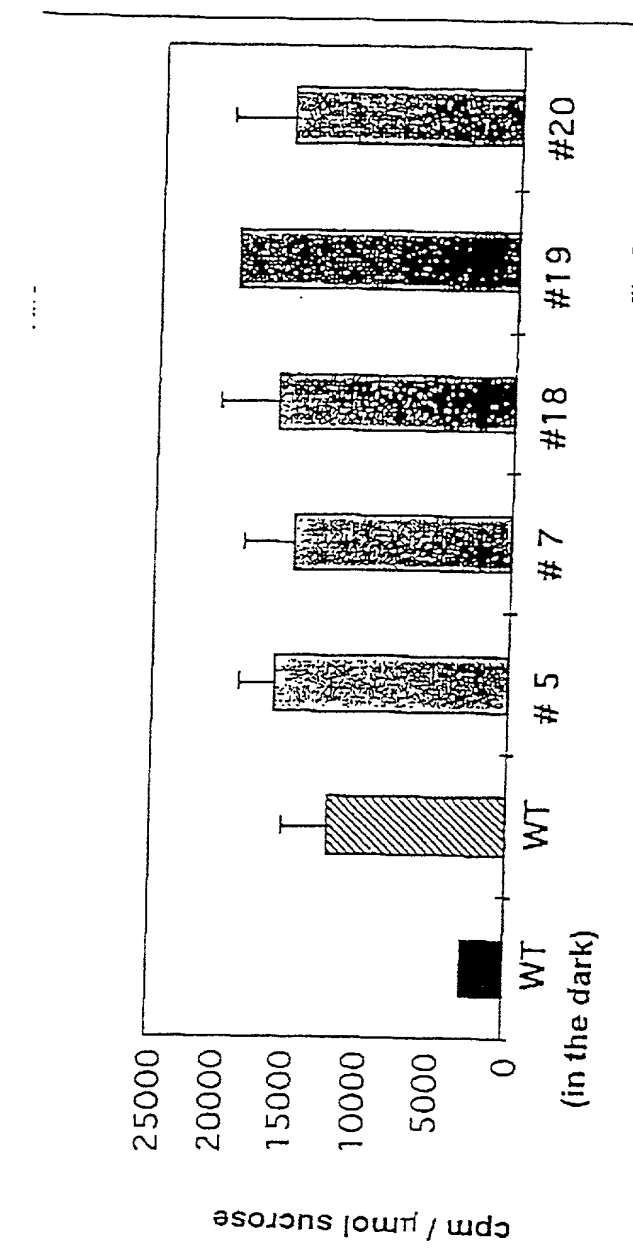


Figure 13

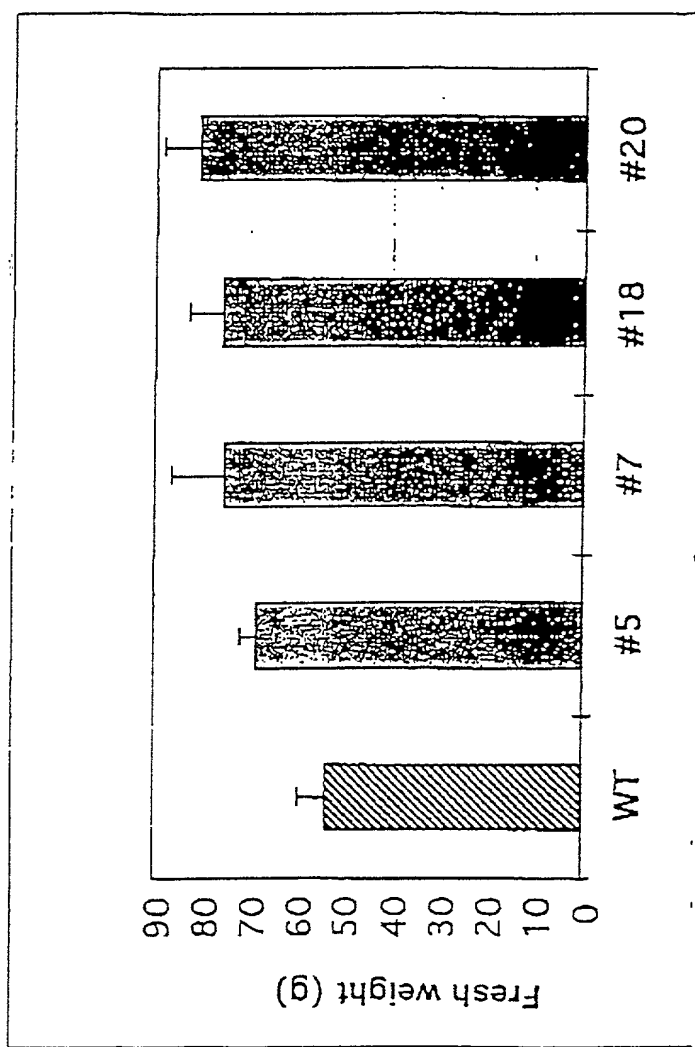


Figure 14

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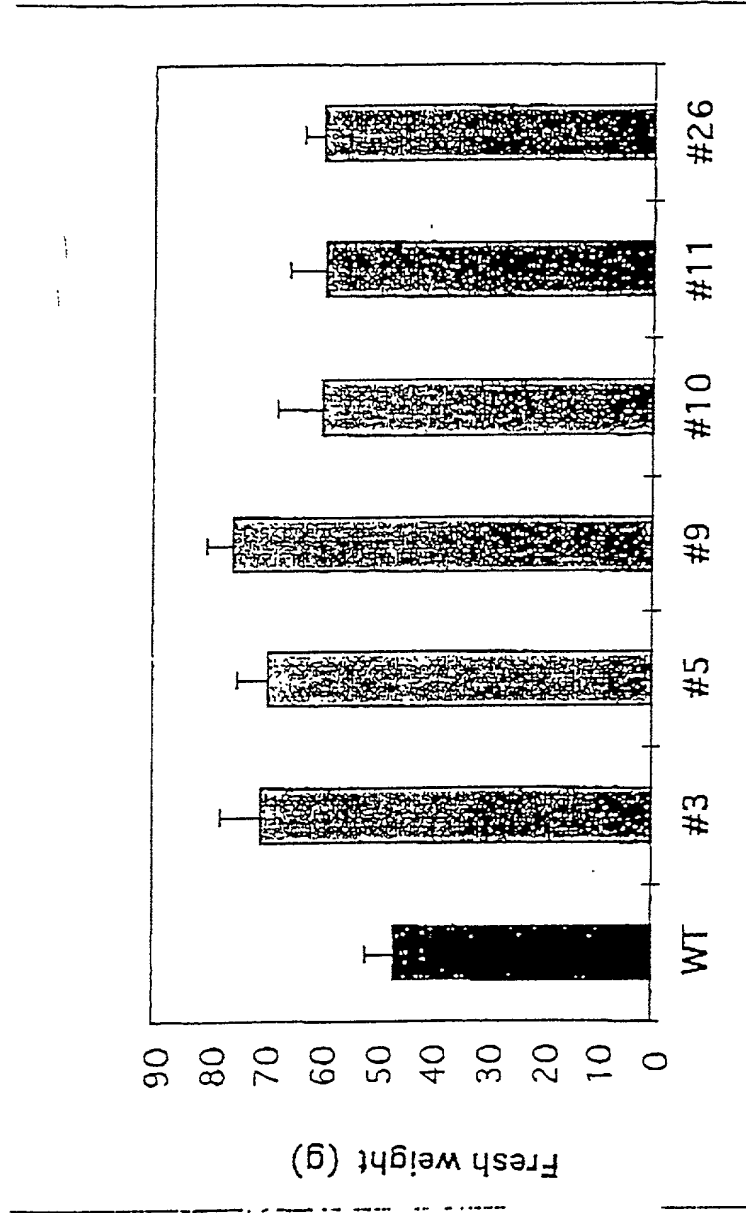


Figure 15

BIRCH, STEWART, KOLASCH & BIRCH, LLPP.O. Box 747 • Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000 • Facsimile: (703) 205-8050**COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT AND DESIGN APPLICATIONS**

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title:

PROCESSES FOR INCREASING THE YIELD IN PLANTSFill in Appropriate
Information -
For Use Without
Specification
Attached:

the specification of which is attached hereto. If not attached hereto,

the specification was filed on February 7, 2000 asUnited States Application Number 09/485,187;

and amended on _____ (if applicable) and/or

the specification was filed on August 5, 1998 as PCTInternational Application Number PCT/EP98/04878; and was

amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)**Priority Claimed**Insert Priority
Information:
(if appropriate)197 34 218.3
(Number)Germany
(Country)August 7, 1997
(Month/Day/Year Filed)☒ ☐
Yes No_____
(Number)_____
(Country)_____
(Month/Day/Year Filed)☐ ☐
Yes No_____
(Number)_____
(Country)_____
(Month/Day/Year Filed)☐ ☐
Yes No_____
(Number)_____
(Country)_____
(Month/Day/Year Filed)☐ ☐
Yes No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below.

Insert Provisional
Application(s):
(if any)_____
(Application Number)_____
(Filing Date)_____
(Application Number)_____
(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Country	Application Number	Date of Filing (Month/Day/Year)
_____	_____	_____
_____	_____	_____

Insert Requested
Information:
(if appropriate)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Insert Prior U.S.
Application(s):
(if any)_____
(Application Number)_____
(Filing Date)_____
(Status - patented, pending, abandoned)_____
(Application Number)_____
(Filing Date)_____
(Status - patented, pending, abandoned)

hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

Raymond C. Stewart	(Reg. No. 21,066)	Terrell C. Birch	(Reg. No. 19,382)
Joseph A. Kolasch	(Reg. No. 22,463)	James M. Slattery	(Reg. No. 28,380)
Bernard L. Sweeney	(Reg. No. 24,448)	Michael K. Mutter	(Reg. No. 29,680)
Charles Gorenstein	(Reg. No. 29,271)	Gerald M. Murphy, Jr.	(Reg. No. 28,977)
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Joe McKinney Muncy	(Reg. No. 32,334)	Donald J. Daley	(Reg. No. 34,313)
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YOU MUST
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FOLLOWING:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First
or Sole Inventor:
Insert Name of
Inventor
Insert Date This
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Insert Residence
Insert Citizenship

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Address

Full Name of Second
Inventor, if any:
see above

Full Name of Third
Inventor, if any:
see above

Full Name of Fourth
Inventor, if any:
see above

Full Name of Fifth
Inventor, if any:
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GIVEN NAME/FAMILY NAME		INVENTOR'S SIGNATURE	DATE*
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GIVEN NAME/FAMILY NAME		INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP	
POST OFFICE ADDRESS (Complete Street Address including City, State & Country)			

533 Rec'd PCT/PTO 11 AUG 2000

PCT #7.
BOX Seq



540

BOX PCT
PATENT

Attorney Docket No. 147-191P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: KWART, Marion et al.
INTERNATIONAL APPL. NO.: PCT/EP98/04878
APPL. NO.: 09/485,187
FILED: February 7, 2000
FOR: PROCESSES FOR INCREASING THE YIELD
IN PLANTS

SUBMISSION OF SEQUENCE LISTING PURSUANT TO
37 C.F.R. 1.825 (e)

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

August 7, 2000

Sir:

In compliance with 37 C.F.R. 1.825(e), enclosed herewith are (1) a paper copy of the SEQUENCE LISTING and (2) a computer disc containing the SEQUENCE LISTING information which is identical to the enclosed paper copy.

Entry of the SEQUENCE LISTING information into the file and favorable action on the merits of the above-identified application are respectfully requested.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By: 

Leonard R. Svensson
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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope to: Commissioner of Patents and Trademarks, Washington

D.C. 20231 on: August 8, 2000
(Date of deposit)

BIRCH, STEWART, KOLASCH & BIRCH, LLP

For M. Silberman
(Signature)

August 8, 2000
(Date of Signature)

SEQUENCE LISTING

<110> Kwart, Marion
Riesmeier, Jorg
Willmitzer, Lothar

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